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PHYTOPATHOLOGY

VOLUME X NUMBER 6

JUNE 1920

# PERONOSPORA IN TURNIP ROOTS

MAX W. GARDNER[1]

WITH PLATE XII

Among turnips in storage and in the local market in Lafayette, Indiana, an occasional root was found in February, 1918, showing a dark discolored internal region extending down into the root from the crown, which appeared in cross section as shown in plate XII, figures A and B. The affected tissue varied in color from a light brown (fig. B) to a distinct black (fig. A) and was firm and quite turgid. One of these affected roots was cut and placed in a damp chamber in the refrigerator. Upon the cut surfaces of the discolored tissue there occurred a luxuriant development of conidiophores and conidia of *Peronospora parasitica.*

Subsequent tests showed that under such conditions the fungus would sporulate abundantly on a freshly cut surface of the affected tissue within twenty-four hours. Upon prolonged incubation in the refrigerator, the sporophores were produced in abundance on what was previously normal tissue surrounding the discolored region. This indicated that the fungus was enlarging its area of invasion. In one case, all of the cut surfaces of a root bore the aerial fungous growth after three weeks incubation, indicating that the infestation of the tissues had proceeded to completion. Such fresh invasion caused only a very light brown mottling and streaking of the tissues, not nearly so marked a discoloration as was found in the freshly collected specimens. In freshly cut specimens there were indications that the cortical tissues were not as readily invaded as the central cylinder (pl. XII, fig. B). The affected tissue was found to be predisposed to rotting by Rhizoctonia and the soft rot bacteria.

Microscopic examination of razor sections of the affected tissue revealed the presence of extremely large, club-shaped haustoria in the host cells. Invaded cells are discolored. From the distribution of these discolored

[1]Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, Lafayette, Indiana.

cells, as well as the gross appearance of the tissue, it is evident that the infection extends in strands of various shapes and sizes interspersed with normal tissue.

In order to ascertain the relation of the parasite to the host tissue, blocks of diseased tissue were fixed and embedded in paraffin. Sections 15 $\mu$ thick stained with safranin, gentian violet, and carbol fuchsin proved very satisfactory. From the rather scanty intercellular mycelium, large, club-shaped, usually dichotomously lobed haustoria are produced within the host cells (pl. XII, figs. C and D). In many instances the haustoria are much branched, and almost or completely fill the lumen of the host cell. No enlargement nor multiplication of host cells is caused. No oospores have been found.

The spores produced on the cut surfaces of the roots in the refrigerator germinated in tap water very readily at refrigerator temperatures of 8° to 12°C., less vigorously at 19°C., and, at 23° to 25°C., only a very low percentage germinated, of which the germ tubes at twenty-four hours were only about one-third as long as those produced at the lower temperatures. At 29°C. no germination occurred.

With a suspension of these spores, a series of atomizer inoculations was made on turnip, rutabaga, and radish seedlings. These plants were held three days in a root cellar at 10°C. and then removed to the greenhouse. In another series the inoculum was rubbed on the cotyledons of turnip seedlings which were incubated in the refrigerator four days and then removed to the greenhouse. At the end of two weeks, five out of twelve turnip seedlings of the last series and two out of eleven turnip seedlings of the first series were infected and showed abundant sporulation of Peronospora on the cotyledons. No infection occurred on the rutabagas and radishes nor on uninoculated turnips and radishes used as controls. An unsuccessful attempt was made to transplant the fungus to a sound turnip root by inserting a block of diseased tissue into a freshly made cavity of similar size and shape which was then sealed with paraffin. Microscopic examination showed that the fungus had not invaded the sound tissue in contact with the inoculum.

Melhus[2] found *Peronospora parasitica* living over winter by means of a perennial mycelium in *Lepidium virginicum*. The occurrence of viable mycelium of this fungus in turnip roots late in the winter indicates that it may also live over winter in turnips which remain in the field.

DEPARTMENT OF BOTANY
PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, INDIANA

[2] Melhus, I. E. Perennial mycelium in species of Peronosporaceae related to Phytophthora infestans. Jour. Agr. Res., **5**: 59–69. Pl. III, 1 fig. 1915.

PLATE

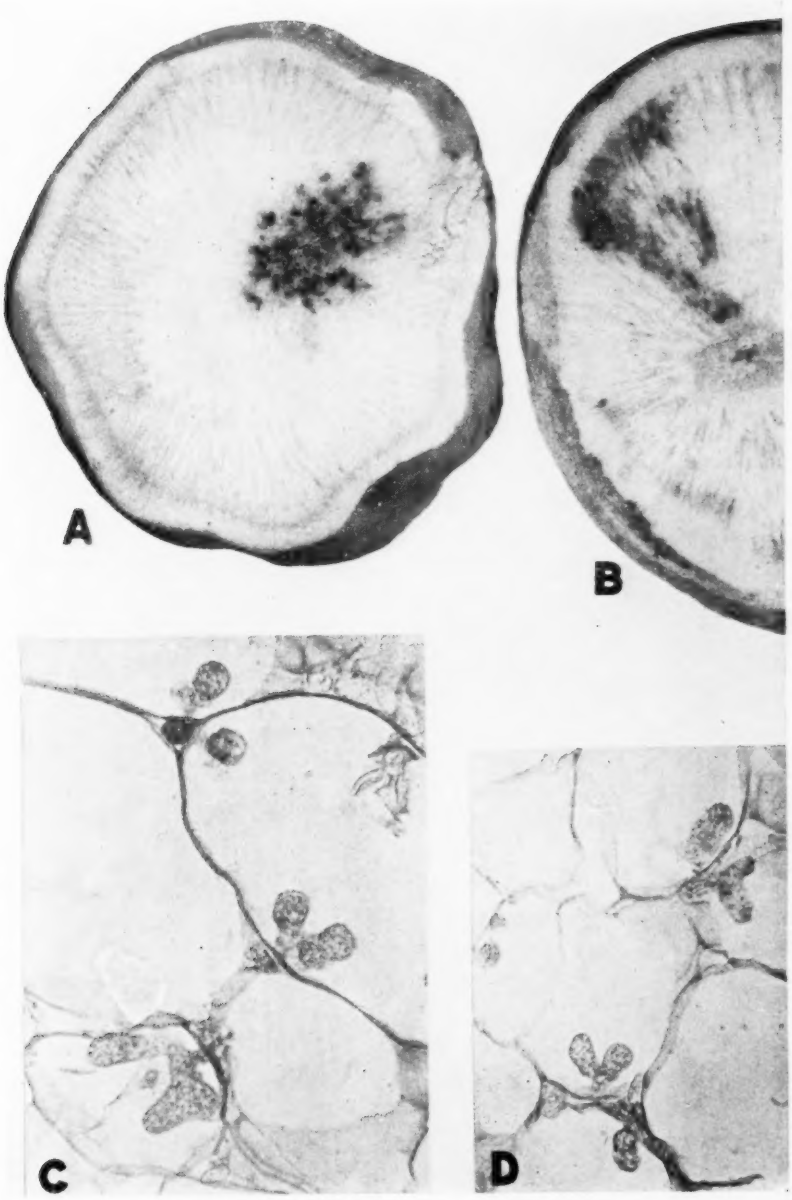
## PLATE XII

FIG. A. Cross section of a turnip showing blackened tissue infected with *Peronospora parasitica.*

FIG. B. Similar to Fig. A, showing another type of lesion, brown in color.

FIG. C. Photomicrograph of a section of turnip root tissue infected with *Peronospora parasitica,* showing the intercellular mycelium and the club-shaped haustoria within the host cells. (Magnified about 280 times.)

FIG. D. Similar to Fig. C. (Magnified about 210 times.)




GARDENER: PERONOSPORA IN TURNIPS

# EXPERIMENTS UPON FORMALDEHYDE-DRIP CONTROL OF ONION SMUT

J. C. WALKER

Since the increasing importance of onion smut (*Urocystis cepulae* Frost) in southeastern Wisconsin has necessitated the use of a disinfectant with the seed in certain areas to insure a crop, the experiments reported herewith were undertaken to determine the most effective means of control. In 1913, when the first experiments were carried out, both the sulphur and lime method described by Sirrine and Stewart[1] and the formaldehyde-drip treatment devised by Selby[2] were tried. That season's results seemed sufficient to show the inferiority of the former method, both as to efficiency in controlling the disease and ease of proper application. Further efforts were directed during the following seasons toward the formaldehyde-drip method. The chief practical objection to the latter has been the additional load on the hand seeder made necessary when the tank containing the disinfectant is attached. Accordingly, one of the chief objects of the work was to determine the maximum concentration and minimum quantity of the solution possible for most effective control of the disease.

## PLAN OF THE EXPERIMENTS

All of the experiments from 1913 to 1916, inclusive, were performed on the same plot of ground. This consisted of an area 475 by 50 feet, selected from a badly diseased field in the Racine, Wisconsin, district. The ground was nearly level, the soil was a uniform, black, sandy loam, and the disease proved to be very evenly distributed. Rows were run lengthwise of the field, and the series of treatments was repeated two or more times in order to compensate as much as possible for any slight variations in the soil. The Red Globe variety of onion was used throughout. Seed was planted one-half to one inch below the surface, by means of a standard Planet Jr. onion seeder, at the rate of 3 to 4 pounds per acre, which is customary in this section for the growing of large bulbs.

[1] Sirrine, F. A., and Stewart, F. C. Experiments on the sulphur-lime treatment for onion smut. N. Y. (Geneva) Agr. Expt. Sta. Bul. 182, p. 143–172. 1900.

[2] Selby, A. D. The prevention of onion smut. Ohio Agr. Expt. Sta. Bul. 131, p. 47–51. 1902.

The drip attachment has been changed from time to time during the course of the experiments, and since the type in present use has been fully described elsewhere,[3] it need not be considered further at this piont.

## DISCUSSION OF RESULTS

Since obviously many factors other than the disease influence the crop in an experiment of this sort, it is not advisable to depend upon yield alone in comparing the efficiency of various treatments. The percentage of smutted seedlings seems to be a much better criterion on which to base judgment in this case. Throughout the experiments the amount of disease was estimated from time to time. This was usually done by measuring off four 20-foot strips crosswise of the plat at equal intervals and counting healthy and smutted plants in these portions of each row. During 1914 and 1915 such counts, made approximately one, two, and three months after planting, showed that in both seasons the maximum amount of disease in all the treated plots was secured at the second count, that is in the last part of June. The decline in the percentage of diseased plants after this date was due to the death of infected seedlings. In the results summarized in table 1, counts made at this time of the season are given, except for 1913, when the first counts were not secured until August.

In 1913, three strengths of formaldehyde were used, namely, ½ liquid ounce of the concentrated solution (37 to 40 per cent) to one gallon of water (1:256), 1 ounce per gallon (1:128), and 2 ounces per gallon (1:64). It is clear from the results given in table 1 that the strongest solution gave the best control, as indicated by both the amount of smut and the final yield, while the weakest solution had practically no effect on the disease.

In 1914, the weakest solution was dropped from the schedule, while a stronger one (1:32) was added, and, in addition, the amount of liquid was varied (118 and 236 gallons per acre). In the case of both quantities the 1:32 solution caused a reduction of stand, and, moreover, the control of the disease was no more complete than in the case of the 1:64 solution. The low yield in the case of the 1:64 solution applied at 118 gallons per acre could not be satisfactorily explained, but it is evidently not due to seedling injury, since the same strength at 236 gallons gave a much higher yield. Considering the percentage of disease, the latter strength at 236 gallons is the best of the series, although the 1:128 solution at 236 gallons per acre and the 1:64 solution at 118 gallons gave nearly as good results.

In 1915, attention was confined to the 1:128 and 1:64 solutions applied at 118 and 236 gallons per acre. As indicated in the previous season,

[3] Walker, J. C. Onion diseases and their control. U. S. D. A. Farmers' Bul. 1060, p. 1–24. 1919.

the disease was controlled much more effectively when the larger quantity was applied, and in this case the weaker solution (1:128) gave as satisfactory results as the stronger (1.64).

In 1916, experiments parallel with those carried out by Dr. I. C. Jagger in New York were planned, and consequently the details of treatment

TABLE 1

*Summary of results of formaldehyde control experiments. Racine, Wisconsin, 1913–1916*

| YEAR | DATE OF SOWING | NUMBER OF PLOTS USED | NUMBER OF ROWS PER PLOT | STRENGTH OF SOLUTION | NUMBER OF GALLONS PER ACRE | AMOUNT OF SMUT | | FINAL YIELD IN BUSHELS PER ACRE |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Date of observation | Per cent | |
| 1913 | April 22 | 5 | 2 | Untreated | | August 6 | 24.0 | 325.2 |
| 1913 | April 22 | 2 | 2 | 1–256 | 118± | August 6 | 27.5 | 340.8 |
| 1913 | April 22 | 2 | 2 | 1–128 | 118± | August 6 | 5.6 | 505.9 |
| 1913 | April 22 | 2 | 2 | **1–64** | **118±** | August 6 | **2.5** | **588.3** |
| 1914 | April 23 | 5 | 2 | Untreated | | June 30 | 52.0 | 86.4 |
| 1914 | April 23 | 2 | 2 | 1–128 | 118± | June 30 | 13.4 | 344.4 |
| 1914 | April 23 | 2 | 2 | 1–64 | 118± | June 30 | 10.3 | 282.9 |
| 1914 | April 23 | 2 | 2 | 1–32 | 118± | June 30 | 11.5 | 215.8 |
| 1914 | April 23 | 2 | 2 | **1–128** | **236±** | June 30 | **10.2** | **295.9** |
| 1914 | April 23 | 2 | 2 | 1–64 | 236± | June 30 | 6.2 | 409.2 |
| 1914 | April 23 | 2 | 2 | 1–32 | 236± | June 30 | 6.3 | 298.8 |
| 1915 | April 14 | 3 | 2 or 4 | Untreated | | June 25 | 52.5 | 400.6 |
| 1915 | April 14 | 2 | 2 or 4 | 1–128 | 118± | June 25 | 28.5 | 633.6 |
| 1915 | April 14 | 2 | 4 | 1–64 | 118± | June 25 | 15.3 | 721.6 |
| 1915 | April 14 | 2 | 4 | **1–128** | **236±** | June 25 | **4.3** | **746.7** |
| 1915 | April 14 | 2 | 4 | 1–64 | 236± | June 25 | 3.5 | 654.2 |
| 1916 | April 24–27 | 10 | 1 | Untreated | | June 19 | 54.9 | 72.9 |
| 1916 | April 24–27 | 3 | 1 | 1–175 | 70–100 | June 19 | 43.1 | 117.1 |
| 1916 | April 24–27 | 4 | 1 | 1–125 | 81–100 | June 19 | 29.0 | 163.8 |
| 1916 | April 24–27 | 4 | 1 | 1–75 | 80–107 | June 19 | 13.7 | 177.5 |
| 1916 | April 24–27 | 4 | 1 | 1–175 | 128–150 | June 19 | 31.4 | 181.8 |
| 1916 | April 24–27 | 4 | 1 | 1–125 | 134–150 | June 19 | 24.2 | 207.4 |
| 1916 | April 24–27 | 4 | 1 | **1–75** | **131–156** | June 19 | **14.6** | **205.3** |
| 1916 | April 24–27 | 3 | 1 | 1–175 | 200–224 | June 19 | 17.7 | 291.5 |
| 1916 | April 24–27 | 4 | 1 | **1–125** | **197–200** | June 19 | *5.8* | *350.8* |
| 1916 | April 24–27 | 4 | 1 | 1–75 | 193–198 | June 19 | 7.1 | 247.0 |

were changed slightly. The desirability of applying larger amounts was again brought out, and when approximately 200 gallons per acre were used, the 1: 125 strength gave the best control. Experiments in which the quantity was increased to more than 236 gallons were not performed, since this represents the approximate limit which can be practically

handled on a common hand seeder, and the control is complete enough for general purposes when this amount is used.

It may be concluded from these data that for the section concerned at least, approximately 200 gallons of solution per acre will give the most satisfactory results, and that there is no advantage in this case in increasing the concentration above 1:128 (1 liquid ounce per gallon or 1 pint to 16 gallons). Where there is serious objection to using this amount of liquid because of difficulty in application, the quantity may be reduced to 120 to 150 gallons, but in this case the concentration should be increased to 1:64 (2 liquid ounces per gallon). Raising the strength of the solution beyond this point, however, entails danger of reduction of stand due to seedling injury.

It is very probable that numerous environmental factors, such as type of soil, temperature, and moisture, influence more or less the effect of formaldehyde upon both the fungus and the host plant. Some of these perhaps have significant practical bearing and should be considered in

TABLE 2

*Results of commercial application of 1:128 formaldehyde for the control of onion smut*

| YEAR | GALLONS PER ACRE | PER CENT PLANTS DISEASED | | YIELD, BUSHELS PER ACRE | |
|---|---|---|---|---|---|
| | | Treated | Untreated | Treated | Untreated |
| 1916 | 130 | 15.0 | 43.5 | 328 | 203 |
| 1917 | 200 | 2.3 | 52.2 | 544 | 200 |
| 1918 | 200 | 2.5 | 50.0 | 682 | 186 |

making general recommendations for a wide area on the basis of experiments in one locality. The conditions following sowing also vary from year to year in the same locality. For instance, there was rainy weather immediately after the experimental plots were planted in 1914, while in 1915 no rain fell for eighteen days after sowing was completed. In the former year there was little or no retardation of seed germination in the treated plots, while in the latter all the treated plots were distinctly slower in starting off than were the checks. Moreover, in 1915, where the smaller quantity of solution was used, the control of the disease was much less pronounced than in similar plots the previous year.

However, it is not believed that this or other factors are influential enough to seriously limit successful control of the disease by this method in the section concerned, and probably the same statement holds for other northern onion-growing regions. As further substantiation of this tentative conclusion, data are cited in table 2 which were taken for a period of three years from a badly diseased field in the Racine district, where a 1:128 formaldehyde solution was applied.

In sowing onion seed for sets, as they are grown in the Wisconsin, Illinois, and Indiana sections by the single row method, about twenty times as much seed is used per row as in the case of large bulb production. This fact does not necessarily alter the quantity of formaldehyde solution to be applied, since the same amount of soil surrounding the seed is to be disinfected in either case. Furthermore, results secured with the use of the above formula by onion set growers in both the Racine and the Chicago districts indicate that it is satisfactory for use with this crop. With the multiple-row system, in use in certain onion sections, a different formula will be necessary.

DEPARTMENT OF PLANT PATHOLOGY
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# A NEW CORN SMUT IN WASHINGTON

B. F. Dana and George L. Zundel

With Four Figures in the Text

*Occurrence.* In the fall of 1919, a smut of corn (*Zea mays* L.) not previously known to occur in Washington was found by the writers in the vicinity of Pullman. The symptoms and microscopic characters of the disease showed that it was not the common or boil smut of corn but was identical with the head smut of sorghum caused by *Sphacelotheca reiliana* (Kühn.) Clinton.

Two widely separated fields were found to be affected, one field showing 40 per cent of smutted plants. "Thayer's yellow dent" corn was the variety grown in each case, the seed having been selected from locally grown stock for a number of years. One grower stated that smut occurred on his corn "every year" but it cannot be said with certainty that head smut occurred before the present season in Washington, either on corn or sorghum, the more common host for this disease. We have no reports of the disease west of Nebraska or Texas. However, a disease having the symptoms of head smut has been seen by one of the writers in Utah on sorghum, although a definite determination of the fungus was not made. The fact that the disease has occurred, as far as is known, only on locally grown stock makes the manner of introduction difficult of explanation.

*Description.* Head smut has received full treatment at the hands of Potter [1] but most attention was given to the disease as it occurs on sorghum. Butler [2] gives a detailed description of head smut on corn as it occurs in India. Below is a somewhat detailed description of head smut on corn as it occurs in Washington. Sori or smut masses were observed in ears and tassels only. In one field tassels were most commonly affected, smutted ears being rare. In the other field the reverse was true.

The majority of the tassels showed black, powdery smut masses, involving more or less of the whole inflorescence (see fig. 1). In a few cases sterility was produced, accompanied by a distinct proliferation of the flower parts and the tassel as a whole. Figure 2 shows this development. The production of leafy shoots in the place of normal parts is clearly shown.

[1] Potter, A. A. Head smut of sorghum and maize. Jour. Agr. Res. **2**: 339–372. 1914.

[2] Butler, E. J. Fungi and disease in plants. Pp. 199–201. 1918.

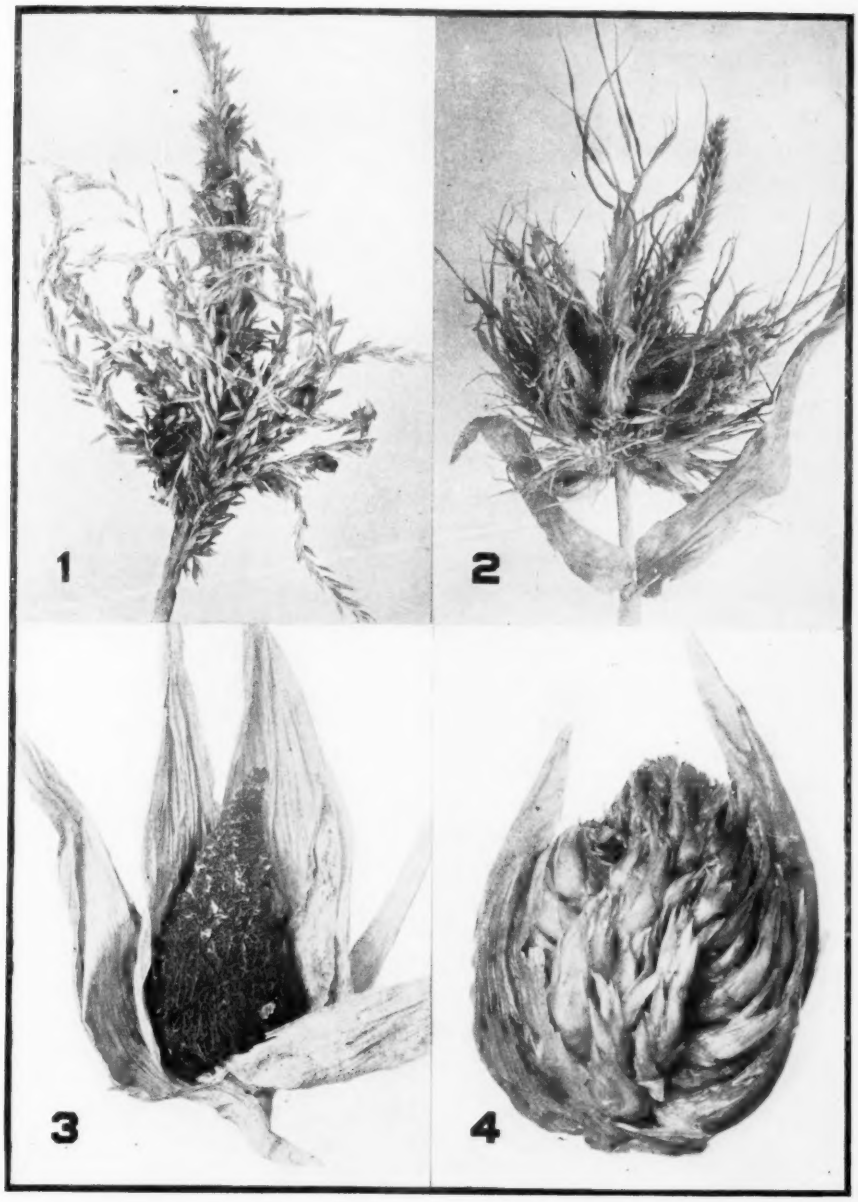


FIG. 1. TASSEL SHOWING THE USUAL EFFECT OF HEAD SMUT
FIG. 2. TASSEL SHOWING STERILITY AND PROLIFERATION
FIG. 3. A SMUTTED EAR SHOWING THE CHARACTERISTIC "FIBERS"
FIG. 4. STERILITY AND PROLIFERATION IN THE EAR

The only smut spores produced in this ear are to be seen in a small mass near the top of the figure.

Within some of these shoots there was a mass of smut spores not apparent from the outside. Figure 3 shows the usual effect observed on the ear. The whole ear is involved and the characteristic "fibers" running through the mass are clearly shown. This character alone would be sufficient to distinguish it from the common smut caused by *Ustilago zeae* (Beckm.) Unger. A very common occurrence was the production on a smutted plant of abortive ears at the nodes below the main ear. These were usually completely smutted, some showing, however, irregular, leafy growth. In one case observed, leafy growth involved the whole ear, as shown in figure 4. The nature of this proliferation is essentially the same as was shown in figure 2 on the tassel. In a small section of one of the leafy shoots there was a small mass of smut spores. This can be seen near the top of figure 4. These figures bring out strikingly the fact that head smut may produce sori in the tassels and ears or may cause abnormal development in these parts. Affected plants were not as tall or thrifty as normal ones, and exhibited a rather uniform dwarfing effect of the fungus. Some instances were observed where a normal ear was produced on a plant showing smut in the tassel. Many plants were seen where the ear was smutted and the tassel was normal; however, in one field where special study of symptoms was made the ear was generally affected where the tassel showed the smut masses. It is hoped that conditions will not be favorable for the spread of head smut in Washington.

AGRICULTURAL EXPERIMENT STATION
PULLMAN, WASHINGTON

# COIX SMUT

C. C. Thomas

With One Figure in the Text

The appearance of a disease, previously unreported in this country, in the Plant Quarantine Greenhouse of the United States Department of Agriculture is of special interest to all who are concerned with the prevention of the introduction of new diseases into this country.

Coix smut (*Ustilago coicis* Bref.) has recently appeared on a plant grown from one lot of seed of *Coix lachryma jobi* sent in by Dr. W. H. Weston of the Office of Cereal Investigations, from Kalasungay, Mindanao, Philippine Islands.

The pathological inspectors of the Federal Horticultural Board ordered this lot of seed grown in the quarantine greenhouse as a precautionary measure, inasmuch as it came from within the territory covered by Quarantine No. 24, which prohibits the importation of *Zea mays* and its relatives from certain Oriental countries, as a protection against the downy mildews of corn.

The seed was planted April 14, 1919, and the plants grew to a height of ten feet before flowering. The disease was first observed December 29, 1919, at which time the plants had almost finished flowering. There were four culms in the pot in which the disease appeared, only one of which was diseased. One culm did not flower, and the flowers on the other two did not set seed.

Our knowledge of the life history and distribution of this disease is very imperfect. It was first described by Brefeld,[1] his specimen having been received from Barclay of India. Butler[2] reports that it has since been collected in Bombay and Java. He further states that no information is available regarding the amount of damage caused by the disease, but that it may cause considerable loss, since it entirely destroys the grain.

There is a specimen of this disease in the Office of Pathological Collections of the United States Department of Agriculture, which was collected by Kusano in Japan in 1904.

Every grain of a diseased head is converted into a black spore mass with a slight increase in size, which in some cases may reach almost twice the

[1] Brefeld, O. Untersuch aus d. gesammtgeb. D. Mykologie, **12**, p. 110, 1895.

[2] Butler, E. G. Fungi and Disease in Plants, p. 242, 1918.

size of the normal grain. The grain may be badly deformed or it may be almost normal in outward appearance, as is shown by the photograph (fig. 1). The spore mass is surrounded by a membrane and is more or less

FIG. 1. SMUTTED HEAD OF COIX LACHRYMA-JOBI

All grains here shown are filled with a black spore mass. Photographed by E. I. Crandall.

hidden by the glumes. The leaves and portions of the culms adjoining the diseased grains are distorted and bent. The smut also appears on the culm, as is shown very distinctly by the photograph (fig. 1).

The spores are dark brown, round or slightly oval, and have an echinulate epispore. Measurements of the spores in this material correspond very closely to those given by Brefeld (7–9$\mu$).

The spores have been germinated without difficulty on ordinary blotting paper moistened with tap water and placed in a moist chamber. A typical four-celled promycelium is produced, and terminal and lateral sporidia are produced, which in turn may bud off secondary conidia. In some cases the primary sporidia elongate into a septate filament which buds off secondary conidia, terminally or laterally, near the septa.

Brefeld describes the germination of the spores after being kept in moist earth two years and then sown in a nutrient solution, but he failed to get germination in water. Butler reports the germination of the spores in Java in water and in a nutrient solution immediately after ripening.

The life history of the causal organism is unknown and it would be difficult to say in this case whether it was introduced by spores on the seeds or by dormant mycelium within.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D. C.

# ABNORMAL GROWTH INDUCED BY CHLORAL HYDRATE SOIL TREATMENT

CARL HARTLEY

WITH ONE FIGURE IN THE TEXT

In soil-treatment experiments conducted by the writer and Mr. S. C. Bruner in western Kansas in 1914, primarily for the purpose of securing data on the damping-off disease, seedlings of western yellow pine (*Pinus ponderosa*) exhibited an unexpected reaction. A number of plots were sown in a very light, sandy soil containing little humus and practically neutral as shown by hydrogen-ion determinations by Dr. L. J. Gillespie. Two of these plots were treated with chloral hydrate in quantities of $\frac{1}{4}$ and $\frac{9}{128}$ ounces per square foot, respectively (0.076 and 0.172 kgm. per square meter). The applications were made in aqueous solution three days before sowing. A fair number of seedlings appeared above ground in all of the plots in this series by seventeen days after the seed was sown. In the five untreated plots, and in plots treated with dried blood, bone meal, ether, carbon bisulfid, sulfuric acid, formaldehyde, methyl alcohol, and ethyl alcohol, the seedlings were normal in appearance. In the chloral hydrate plots the number of seedlings appearing was only three-fifths as great as in the two nearest untreated plots (sown with equal quantities of seed and from the same lot) and many of the seedlings were strikingly abnormal in size and form. Typically abnormal seedlings from these plots are shown in figure 1, *A* and *B*; figure 1, *C*, shows a seedling of normal type for comparison. The affected seedlings were taller than normal plants, and the cotyledons and the first juvenile needles were partially fastened together, forming imperfect cylinders. The cotyledons were longer than those of normal seedlings. The loss from damping off in the chloral hydrate plots was distinctly heavier than in the untreated plots—47 per cent of the seedlings against 25 per cent in the two nearest untreated plots.

The size and form of pine seedlings during the first few weeks after germination are relatively constant. Hundreds of seedbeds of *P. ponderosa* and other conifers have been observed in different parts of the country, and following soil treatments with acids, hydroxides, salts, and organic substances. In no other case was so abnormal a development found. Occasional seedlings of both *P. ponderosa* and *P. resinosa* germinated in autoclaved soil containing compost have been found with adhesions be-

tween two or three cotyledons, which do not break up until two or three weeks after the seedlings appear. In the autoclaved soil as in the chloral hydrate plots the seedlings showing the adhesions were generally above normal size. The adherent epidermal surfaces after separation appeared normal.

It is of course impossible to say whether the striking abnormality in the chloral hydrate plots was due to a direct effect of the chloral hydrate

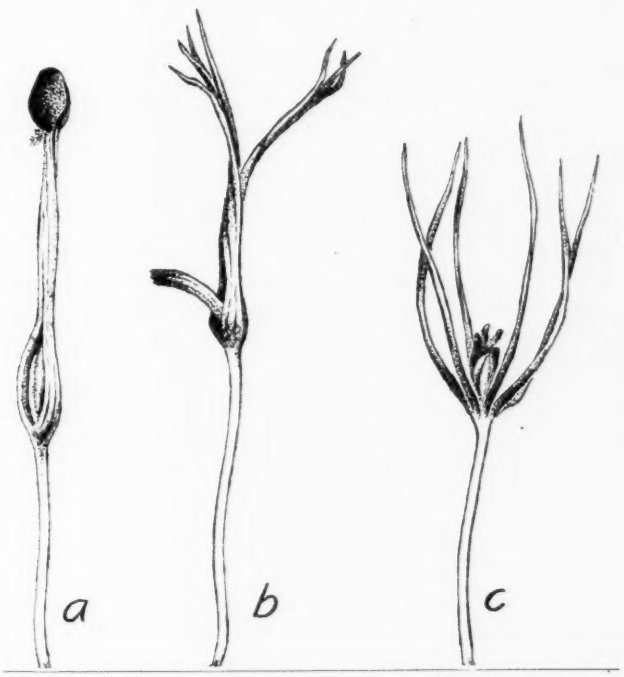


FIG. 1. SEEDLINGS OF PINUS PONDEROSA

*A* and *B*, from a seed bed which has been treated with chloral hydrate; *C*, normal type from a neighboring untreated bed. Natural size.

or one of its derivatives on the seedlings, or to some indirect effect. The fact that the tests were in a coarse mineral soil suggests the possibility of a direct effect. In both the chloral hydrate plots and the autoclaved soil-compost mixture the amount of soluble organic matter was presumably above average.

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# A BATTERY OF CHAMBERS WITH DIFFERENT AUTOMATICALLY MAINTAINED TEMPERATURES

B. E. Livingston and H. S. Fawcett

ABSTRACT

This paper calls attention to the need for a number of maintained temperature chambers that may be operated simultaneously, for studies dealing with the relations of temperature to growth, etc. The main essentials of a battery of seven such chambers, constructed in 1916–1917 at the Laboratory of Plant Physiology of the Johns Hopkins University, are described. A suitable rectangular tank is divided into ten compartments, the first being for ice and water and the second and tenth being for water, with automatically controlled electric heaters and stirrers. The seven intervening compartments contain the cylindrical culture chambers, each supplied with water-jacket and stirrer. Water does not circulate from one compartment to another, but the water of each compartment (excepting the first) is mechanically stirred. Access to the chambers is had from above and the motor-driven stirring mechanisms and heating units are connected below. Suitable insulation and an outer case for the whole series are provided.

In operation, the water of one heated compartment is kept at a lower temperature and that of the other heated compartment is kept at a higher temperature; the seven intervening culture chambers assume and maintain different temperatures between the two selected extremes. One setting had the two controlled temperatures fixed at 5° and 45°C., respectively, and the maintained temperatures of the culture chambers were 7.5°, 13.5°, 18.5°, 24.5°, 30°, 35.5°, and 40°C. After a preliminary period of less than 48 hours, during which a steady state is established, the apparatus operates indefinitely, with fluctuations in any chamber of not over 0.5°C. A table of growth rates of the fungus *Phytophthora terrestria* on cornmeal agar for the seven maintained temperatures just mentioned, is appended.

* * * * * *

The experimental study of the influence of temperature upon physiological processes requires a number of distinct chambers, each with its own controlled temperature, so that a whole series of different temperature conditions may be tested simultaneously. This need has been felt especially in connection with certain lines of study in pathology and bacteriology, as well as in many lines of physiology. Numerous makes of chambers, or incubators, with automatically controlled temperature have made their appearance on the apparatus market during the last decade, while other devices have been specially constructed in various laboratories; but considerations of expense have thus far generally prevented the simultaneous use of a considerable number of these. Aside from the matter of cost, moreover, a really satisfactory incubator usually occupies so much

space that the room needed for a battery of them is frequently in default. On the whole, it may be said that, up to the present time, there are comparatively few laboratories for pathological or physiological experimentation in which as many as five chambers with reliably controlled temperature may be brought simultaneously into service.

Being impressed by the very great need for a much more thorough understanding of the physiological relations of temperature than is now available, and being convinced that productive enquiry into this fundamental field of physiological science requires, in the very first place, such a battery of temperature-controlled chambers as has just been suggested, we have attempted to satisfy our needs by constructing a series of these in the Laboratory of Plant Physiology of the Johns Hopkins University. This paper deals with some of the essentials of their construction and operation.

Chambers with temperature control may be classified primarily in two categories: (1) those in which light is allowed to act on the experimental organisms or material, and (2) those from which light is excluded. Obviously the second kind offers far less practical difficulty than the first, and the chambers to be described below were planned as dark chambers. Each of these two categories of chambers may be further subdivided into two general classes: (a) those in which the selected temperature is *maintained* for long periods of time, and (b) those in which a definite rate of temperature change is provided for by automatic controls. So far as we are aware the latter kind of apparatus has not yet been seriously attempted, though it will become essential as soon as the influence of *rate of temperature change* upon organisms begins to attract attention. Our apparatus belongs essentially to the former class. It was our aim, then, to devise and construct a battery of dark chambers, in each of which the temperature would be automatically maintained for any desired length of period. The series was to be as compact as possible, and access to each chamber was to be independent and as easy as might be. The size of chamber chosen was such that the smallest form of thermograph now on the market (the one taking record sheets 22 × 6.5 cm., made by J. Richard, Paris) might operate within the chamber and still leave plenty of room for cultures, etc.; it is highly desirable that every thermostatic chamber have its own individual thermograph. Since the consensus of experience in temperature maintenance points clearly to the employment of the water-bath type of chamber, on account of the enormous specific heat of water and the ease with which temperature differences within the same chamber may be avoided by circulating this liquid, each of our chambers is surrounded below and laterally by a considerable mass of continuously circulating water. Access to the chambers is had from above, which fact, together with other considerations, has made it impracticable to water-jacket the tops. Ex-

perience has also shown that the electric current is by far the most satisfactory source of heat for such temperature control as is here desired, and this was adopted. From our own experience, and that of many others, we determined to employ mercury-contact sensitive members in our thermostats,[1] and the type actually employed, with perfect satisfaction, is that devised by Hasselbring and manufactured by H. J. Green, Brooklyn, N. Y. These beautiful and efficient little instruments may be set to hold any temperature that may be desired in biological work, and they appear to operate indefinitely without any alteration, so that they require no attention after they have been once adjusted. This type of sensitive member far surpasses many other forms that have been described, in precision of operation and in prolonged reliability. Since our aim was to attain the desired end with as little expense as possible, and since the particular *degrees* of temperature (on any particular thermometer scale) represented in such a series of chambers are generally of no practical imimportance in experimentation—temperature being a *continuous* variable—we have employed only two thermostats for the entire battery. The temperatures of the two ends of the series are alone thermostatically controlled, and the intermediate chambers assume temperatures determined by the temperature-gradient that exists between the two ends. Finally, it is generally necessary to employ some temperatures markedly below that of the air surrounding the apparatus, so that some form of heat-absorber is requisite. We have employed the primitive method of allowing melting ice to absorb the superfluous heat at the cold end of the series.

The foundation of the apparatus is a rectangular tank of galvanized sheet-iron 4.2 m. (15 feet, 9½ inches) long, 51 cm. (20 inches) high, and 46 cm. (18 inches) wide, divided into ten conpartments by cross-partitions of the same material. The compartment at one end is the ice tank, large enough to hold ice for a run of a day or two. The second and tenth (last) compartments are small, being only 23 cm. (8 inches) in length, measured on the axis of the large tank. Each of these is supplied with two double stirrers and with a thermostat. The intervening seven compartments are

[1] The word *thermostat* is loosely used by many writers, to denote either (1) the sensitive member of the temperature-controlling system, (2) the whole apparatus, including the chamber walls, the controlling arrangement and the chamber space, or (3) the chamber space alone. We here employ the word in neither one of these three senses, but use it to denote the complete system that controls the heat supply. In this sense the thermostat consists essentially of three parts, the sensitive member, the heating member and the mechanism through which the former controls the heat supplied by the latter. In this sense the word becomes parallel with *klinostat* and *heliostat*, meaning the whole mechanism by which the condition considered is maintained.

each 43 cm. (17 inches) in length, being nearly square as seen from above. Each of them is provided with an internal, vertically placed, galvanized sheet-iron cylinder closed at the bottom and raised about 6 cm. (2½ inches) above the floor of its compartment. A suitable stirring mechanism is introduced through the bottom of each of these cylinders, and two stirring-rods extend upward close to the lateral wall, being bent (in inverted U-form) so as to clear the free edge and then extend downward, on the outside, as far as the bottom of the cylinder. The outer limbs of these rods are continually moving around the cylinder, circulating the water of the compartment. The water-jacket continues beneath the cylinder, so that the latter is completely surrounded by water, excepting for its open top. The whole large tank is suitably encased and is insulated with a 6.5-cm. (2½ inches) layer of plasterer's hair at sides, ends and bottom. Each compartment has a removable top, built up of several layers of thin wood and wool felt, and each of the seven culture compartments (third to ninth, inclusive) has a circular opening in its top through which is suspended a vertical lining cylinder (of multi-perforated, tinned sheet-iron), which is within the cylinder already mentioned, reaching nearly to the bottom of the latter. This lining cylinder has a bottom of perforated material but it is freely open at the top. Around and below it is air-space, and the inner limbs of the stirrer rods move between the lining and the outer cylinder, thus tending to keep the air of the culture chamber (within the lining chamber) in slow but constant motion. The lining cylinders are provided with suitable removable lids, for easy access to their interiors. Cultures, etc., are placed on wire racks fitting in the chambers.

The amount of water used is a very important feature in determining the constancy of the temperatures within the several chambers. The ice compartment holds about 138 liters (39 gallons), each electrically heated compartment holds about 36 liters (10 gallons), and the water-jacket space about each culture chamber holds 39.5 liters (11 gallons) of water, when all are filled to the water level determined by the overflow openings.

All stirrers are operated by a single electric motor, by means of belts and pulleys underneath the whole apparatus and thus entirely out of the way.

As has been said, thermostatic control is applied only at the two ends of the series of chambers, in the two small compartments (second and tenth), the water of each one of these two compartments being held at its own pre-determined temperature. Suitable relays are employed for the thermostats. The maintained temperature of the first directly controlled compartment is somewhat higher than that of the ice compartment. Ice is added at intervals, usually daily. The other controlled compartment (tenth) has a temperature higher than that of the one next to the ice.

Since the principle upon which this battery of chambers is constructed is that of a maintained temperature gradient between the two ends of the series, it follows that constancy in the several chamber temperatures depends upon the preliminary establishing of a dynamic heat equilibrium between the two heated compartments. It also depends, of course, on the constancy of the rate of loss or gain of heat laterally, upward and downward, through the walls of the series. These latter rates are kept nearly constant by making them as small as possible, through insulation.

When the apparatus is started, or when a change in the temperature range has been made, it requires about 24 hours (never over two days) to establish the permanent temperature gradient, and the system must be operated idle until this is accomplished. After a steady condition of temperature has been attained the apparatus operates indefinitely, with the daily addition of ice, and with a total range of temperature fluctuation in any chamber of less than half a degree centigrade.

The following statements are appended as an example, showing how the apparatus may be employed to obtain a growth-temperature curve for a fungus growing on agar plates in petri dishes. The thermostat of the compartment next to the ice compartment was set to maintain a temperature of 5°C., and the thermostat of the other heated compartment was adjusted for a temperature of 45°C. In less than 48 hours the intervening culture chambers had each assumed a practically steady temperature, as shown by the thermograph records. These temperatures were maintained, with a total range of not above 0.5°C., during a period of six days. Petri dish cultures of the fungus *Phytophthora terrestria* on corn-meal agar showed the following average daily radial growth rates (mm.) in the seven chambers respectively.

| | MAINTAINED TEMPERATURE | | | | | | |
|---|---|---|---|---|---|---|---|
| | 7.5° | 13.5° | 18.5° | 24.5° | 30° | 35.5 | 40 |
| First day | 0.02 | 1.0 | 2.5 | 4.2 | 5.5 | 4.8 | 0.0 |
| Second day | 0.15 | 2.0 | 6.3 | 10.5 | 13.8 | 4.2 | 0.0 |
| Third day | 0.21 | 2.3 | 6.4 | 11.3 | 13.3 | 2.6 | 0.0 |
| Fourth day | 0.70 | 3.2 | 8.2 | 11.4 | 13.2 | 2.5 | 0.0 |

It is seen that the minimum maintained temperature for growth was not encountered for any day of the period, but it is evident that this minimum would not have been much below 7.5°, especially for the first day. The maximum for growth clearly lay between 35.5° and 40° for all four days, apparently becoming lower with succeeding days. The optimum temperature for the growth of this organism, as here dealt with, may be considered as about 30°.

## REVIEWS

*Les Vanillieres de Tahiti et Moorea* is the subject of a 44-page report by Dr. E. P. Meinecke, published for the Chamber of Agriculture of these islands in 1916.

In the introduction it is noted that at least twenty-five fungous parasites have been reported on vanilla, many of these occurring in Tahiti and Moorea. The plantations of these islands were reported to be in a wretched condition as shown in the following: In 80 per cent of the fields, the plants were weakened by the over-production of the pods; in 90 per cent, the plants were derived from exhausted vines; 70 per cent of the plantings were more or less ruined by the chickens, the pigs and the horses; 80 per cent of the plantations suffered from too much sun or too much shade; in 90 per cent of the plants were wounds caused by the laborers; in 60 per cent, protection from the sun was neglected.

Vanilla plants grown under such conditions as outlined in this introduction wou. d seem to be subjects proper for a horticulturist rather than for a phytopathologist, but Dr. Meinecke has treated the subject entirely in a practical way. In the first chapter he describes in detail the needs of the vanilla plant or the proper environment for it in order to avoid diseases. In the second chapter he first treats of sun scald, degeneration of the plant, and injuries, or in other words such injuries as would not happen if the plants were grown under proper conditions; then of the insects, none of which seem to be serious, but included are an aspidiotus on the leaves, stem and young fruits, a wooly plant louse (*Pucerons lanigeres*) upon the vine and upon the petioles, ants, a microlepidoptera, a curculionid, and others of less importance The parasitic diseases are then discussed; these include Cuscuta, common but not serious, the anthracnose, the most serious of all and due to *Gloeosporium vanillae* Massee, connected with other forms and given other names also but these mycological questions are not settled. The anthracnose attacks leaves, stems and fruit but does not seriously injure vanilla grown under the most favorable conditions. The author, however, does not go into detail as to what conditions are most favorable for the fungus, nor as to resistant varieties. He mentions Fusarium as attacking the young growth, and speaks of bacteria associated with the rot of the bark and leaves. He did not find *Uromyces joffrini*, *Seuratia coffeicola*, nor *Seuratia vanillae*, which had been reported from Tahiti. The Capnodiaceae are mentioned.

Under the third chapter, which is on the control of the diseases, is discussed spraying, but the author appears to consider it impracticable.

Under chapter 4 on the Culture of the Vanilla, is described the amount of shade or sunlight desirable, the renewal of the plants, the selection and planting of the cuttings, nurseries, layering, distance of planting, care of the vines, and fertilizing of the flowers

Doubtless the object of the paper is attained in emphasizing the necessity of certain conditions for the growth of the plant. It is certain that this is of prime importance. It is, however, to be regretted that more specific information was not obtained as to the specific pests and diseases which attack this plant.

JOHN R. JOHNSTON.

## PHYTOPATHOLOGICAL NOTES

*A seed-borne Sclerotium and its relation to a rice-seedling disease.* A seedling blight of rice that developed in the plats of the rice experiment station at Crowley, La., in 1914, was found by Dr. H. B. Humphrey to be due to an organism belonging to the *Mycelia sterilia* group of fungi. It was characterized by very small black sclerotia and a white sterile mycelium. In culture the fungus closely resembled the Sclerotium mentioned by Godfrey[1] as the cause of a leaf disease of rice, but no relationship between the two has been established. Later studies by the writer showed the seedling-disease fungus to be associated constantly with rice seed grown in Louisiana, particularly with the varieties Honduras and Blue Rose. Honduras rice grown in California, however, did not carry the fungus on the seed even though the original California sowings were made with Louisiana seed. In a single experiment, the fungus could not be isolated from Louisiana seed one year old, thus indicating that the fungus died during the course of the year. Seeds of several varieties from a lot of Chinese rice exhibited at the San Francisco Exposition in 1915 carried what appeared to be the same fungus.

The fungus under consideration was found not only upon the surface of the seed but embedded within and beneath the seed coat in the form of minute sclerotia. Seedling plants from surface-sterilized infected seed grown in sterile culture tubes quickly succumbed to the effects of the fungus, while plants from similarly treated non-infected seed would remain green for weeks. Clean plants grown in pure culture in tubes, inoculated with the fungus at the crown or roots, showed the same effects as those grown from naturally infected seed. Plants grown in pots and flats in the greenhouse were less susceptible to attack by the fungus than those

[1] Godfrey, G. H. Preliminary notes on an heretofore unreported leaf disease of rice. Phytopathology 6:97. 1916.

grown in the laboratory. Evidently the greenhouse conditions were more favorable to plant growth and less favorable to the fungus, while in culture tubes the conditions were exactly the reverse. Such exact requirement on the part of the fungus for favorable conditions for development in order to do serious damage was repeatedly observed in various circumstances. However, many sowings of naturally infected and artificially inoculated seed in the greenhouse resulted either in the production of weak plants, from which reisolations were made, or in failure to produce plants at all.

It was found that Jensen's modified hot-water treatment, namely, soaking in cold water for four hours and then treating with water at 54°C. for ten minutes, was capable of killing the fungus in hulled seed without materially injuring germination of the seed. This indicates the possibility of using some specially adapted modification of the hot-water treatment as a means of securing disease-free rice seed.

Owing to a change of duties the writer was obliged to discontinue further work on rice diseases. All notes on this, as well as on straighthead and other rice diseases, were turned over to his successor, Dr. W. H. Tisdale.

G. H. GODFREY.

*Errata.* Dr. F. D. Fromme calls attention to two important errors which appeared in the dates of dust applications in bulletin 223 of the Virginia Agricultural Experiment Station on "Dusting Experiments in Peach and Apple Orchards."

The date March 23, on page 5, should be April 23.

The date May 8 should be inserted in the Schedule of Applications on page 12.

Mr. F. V. Rand calls attention to two errors in the article on "Some Insect Relations to Bacillus tracheiphilus, Erw. Sm," published in PHYTOPATHOLOGY for March, 1920.

On page 133 the footnote reference in line 5 should be 3.

On page 138, in lines 7 and 36, December should be November.

*Personals.* Dr. Pier Andrea Saccardo, the distinguished mycologist and Professor Emeritus of the Royal University of Padua, Italy, died on the twelfth day of February, in the seventy-fourth year of his age. Professor Saccardo was a member of numerous academies and societies, both Italian and foreign, and is of course known to all pathologists and mycologists by his great Sylloge Fungorum, a compilation intended to include descriptions of all the published species of fungi of the world.

Dr. E. T. Bartholomew has resigned as assistant professor of Botany at the University of Wisconsin to accept a position as assistant professor in Plant Pathology, in the Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

# LITERATURE ON PLANT DISEASES[1]

COMPILED BY ALICE C. ATWOOD AND FLORENCE P. SMITH, BIBLIOGRAPHICAL ASSISTANTS, BUREAU OF PLANT INDUSTRY

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[1] This list includes references, both American and foreign, to the literature of plant pathology and mycology of interest to pathologists. Foreign references published since January 1, 1917, have been included beginning with the list appearing in v. 7, no. 3, June, 1917.

All authors are urged to cooperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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# APPENDIX

## REPORT OF THE MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, PACIFIC DIVISION, HELD AT PORTLAND, OREGON, APRIL 25 AND 26, 1919

The meetings were held at the Imperial Hotel following the Pine Blister Rust Conference for the West, held during the two days preceding at the same place. All of the meetings were well attended, the register showing forty names of persons from Ontario, British Columbia, Washington, Oregon, Idaho, Utah, and California, as well as a number of pathologists from Washington, D. C., and other parts of the country.

AMERICAN PHYTOPATHOLOGICAL SOCIETY
PACIFIC DIVISION

Friday and Saturday, April 25 and 26, 1919

PROGRAM

Friday, April 25

*Morning Session*—9.30 a.m.

TREE DISEASES

Problems in forest pathology. Haven Metcalf.
Peridermium harknessii and Cronartium quercuum. Contribution II. E. P. Meinecke.
The oak fungus disease and orchard wood decay. W. T. Horne.
Round Table discussion of tree diseases. Informal notes from various members.
Some species of Uredinales with many aecial hosts. Ellsworth Bethel.
*Afternoon Session*—2.00 p.m.

DISEASES OF TRUCK CROPS

Some studies of Verticillium wilt of potatoes. M. B. McKay.
Notes on three cabbage leaf spots in the San Francisco Bay market gardens. Elizabeth H. Smith.
Observations on the bacterial blight of field and garden peas in Montana.—Read by title. H. M. Jennison.
Some fungi found in Utah soils. G. L. Zundel.
Open discussion on miscellaneous diseases of truck crops.

Saturday, April 26

*Morning Session*—9.30 a.m.

CEREAL DISEASES

Roll Call.
Studies on the injurious effects of seed treatment on different varieties of wheat in Oregon. Jessie P. Rose.
Some preliminary physiological studies on formaldehyde treated wheat. W. M. Atwood.
Limitations of formaldehyde as a fungicide for seed wheat. Anna M. Hurd.
Seed injury from fungicides and saprophytes determined by the physical conditions of the seed coats. Anna M. Hurd.
The distribution of the two species of stinking smut of wheat in Oregon. Jessie P. Rose.
The distribution of the species of wheat and oat smut in Washington, Idaho, and Utah. G. L. Zundel.
The smut shower of Washington. G. L. Zundel.
The effectiveness of the Haskell method in the treatment of oats and wheat. F. D. Heald.
Some experiments with the covered smut of oats. F. D. Heald.
A preliminary note on the foot rot of cereals in western Washington. B. F. Dana.
The black chaff disease of wheat in Montana—Read by title. H. M. Jennison.
Open discussion on miscellaneous diseases of cereals.
Business meeting of the Society.
*Afternoon Session*—2.00 p.m.
The use of corrosive sublimate in treating seed potatoes. G. R. Hill.
Some experiments on the control of onion smut in Oregon. H. P. Barss.
Dusting versus spraying for disease control in the Hood River Valley. LeRoy Childs.
New dusting materials. R. E. Smith.
Blackheart of apricots. Helen L. Czarnecki.
Studies on the Sclerotinia of apricots. Edith H. Phillips.
Artificial infections of pear and apple blight through the leaves. F. D. Heald.
Blight resistance in Pyrus. F. C. Reimer.
Some results with disinfectants in blight control work. F. C. Reimer.
Pear blight control in the Rogue River Valley. C. C. Cate.
A new dormant spray for San Jose scale, blister mite, aphis eggs, etc. C. C. Cate.
Spraying for apple anthracnose. Arthur Frank.
Open discussion on miscellaneous fruit diseases and their control.

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